Factors Affecting the Development of Cavity Spot of Carrot

E. VIVODA, Graduate Student, R. M. DAVIS, Extension Specialist, and J. J. NUÑEZ, Staff Research Associate, Department of Plant Pathology, University of California, Davis 95616, and J. P. GUERARD, Farm Advisor, 1031 Mt. Vernon Avenue, Bakersfield, CA 93307

ABSTRACT

Pythium violae and P. ultimum, isolated from cavity spot lesions on carrots produced in California, caused typical cavity spots on carrots grown in artificially infested soil in the greenhouse. In a 2 yr survey of 54 fields in the San Joaquin Valley of California, cavity spot incidence was not correlated with soil pH, electrical conductivity, moisture-holding capacity, organic matter, total and exchangeable calcium, particle size distribution, or planting densities (57, 115, or 230 carrots per meter of row). In a growth chamber maintained at 20 C, six commonly grown carrot cultivars were susceptible to both Pythium spp. Five-month-old carrots inoculated with either P. violae or P. ultimum developed about twice the number of lesions that developed on 3- or 4-mo-old carrots. Both pathogens were more virulent at 15 C than at 20 or 25 C. The number of cavity spot lesions per carrot was greater in soils continuously flooded for 24 or 48 hr than on carrots grown in nonflooded soil. P. violae was more virulent than P. ultimum in all experiments.

Cavity spot is a severe disease of carrots (Daucus carota L.) in the San Joaquin Valley of California, where more than 10,000 ha are planted annually. Occasionally, entire fields are abandoned because of a high incidence of the disease. Currently, there are no control measures.

The disease is characterized by root lesions that are elliptical (oriented across the breadth of the root), generally less than 2 cm in length, and about 1-5 mm in depth. Lesions may be present over the entire carrot taproot, but they usually tend to be concentrated on the upper third. There are no visible symptoms on the aboveground part of the plant.

In 1961, cavity spot was reported to be a physiological disorder induced by a deficiency of calcium (4,10). Since then, it has also been attributed to anaerobic, pectolytic bacteria (13), Rhizoctonia (11), and stress from flooding and high soil temperatures (15). A major breakthrough in understanding cavity spot etiology occurred in 1984 and 1985 with reports from Norway and England that fungi-cides specific against oomycetes reduced the incidence of the disease (2,9). Further investigations in England revealed that Pythium species, especially P. violae Cheysters and C. J. Hickman, were associated with cavity spot lesions (3,19).

The objectives of this research were to determine the cause of cavity spot in the San Joaquin Valley of California and the distribution of the disease in relation to a number of soil characteristics, and to examine the effects of carrot cultivar, carrot age, soil temperature, soil moisture, and planting density on cavity spot development.

MATERIALS AND METHODS
Disease survey and methods of isolation. In 1987, soil from 39 randomly selected carrot fields in Kern County, CA, was collected to identify and quantify Pythium species present at the time of planting. Details of the sampling scheme were described previously (8). Five samples of soil (each consisting of five soil cores 2 cm in diameter and 20 cm deep) were collected from each field by sampling the corners and the center of the field. Each sample was diluted (1:25 or 1:50, w/v) in 0.2% water agar and 1 ml was pipetted on PVP agar plates (100 mg of pimaricin, 250 mg of vancomycin, 50 mg of penicillin, 100 mg of pentachloronitrobenzene, and 17 g of cornmeal agar [CMA] in 1 L of distilled water) or modified PARP (10 mg of pimaricin, 250 mg of ampicillin, 10 mg of rifampicin, 25 mg of pentachloronitrobenzene, and 17 g of CMA in 1 L of distilled water) (7) and incubated in the dark for 36 hr. More than 300 isolates of Pythium spp. were recovered, transferred, and maintained on CMA. Identification of Pythium isolates was made from morphological characteristics of cultures grown at 20 C in the dark on potato-carrot agar (20 g each of macerated potatoes and carrots and 20 g of agar in 1 L of distilled water) and on leaf blades of autolysed grass (Poa annua L.) in sterile water (17). A direct baiting technique was also used to isolate fungi from naturally infested soil. For this method, carrot and alfalfa seeds were planted into naturally infested soil in plastic pots (5 x 5 x 5 cm) placed in a greenhouse at 20-30 C. After 2 and 4 wk, the roots were lifted, washed, plated on PARP, and incubated at 20 C in the dark for 24 or 48 hr.

After fungal isolations were completed, undiluted soil samples from each field were bulked and analyzed by the Soil, Water, and Plant Analysis Laboratory, Cooperative Extension, University of California, Davis, for soil pH, electrical conductivity, organic matter, particle size distribution, total and exchangeable calcium, and moisture retention at -10 and -1,500 kPa. These procedures were repeated in 1988 for soil samples from 15 fields.

At harvest (January 1988 and 1989), the incidence of cavity spot was determined by randomly sampling approximately 200 carrots from the corners and center of each field. All carrot cultivars included in the survey were Imperator types, with Sierra, Dominator, Pakmor, Goldmine, Seminola, and Fancy Pak accounting for 68% of the fields. The incidence of cavity spot was correlated with the soil factors listed previously.

In 1988, approximately 100 carrots were randomly sampled every 14 days in each of six Kern County fields to evaluate disease development over the duration of the season. The carrots were washed and separated into groups with or without cavity spot lesions.

To isolate potential pathogens from cavity spot lesions, symptomatic carrots collected from 12 randomly selected fields in both years were surface-sterilized by submerging the roots in 0.525% NaOCl for 2 min. Excised tissue from the margin of the lesions was aseptically placed on the selective media described earlier or potato-dextrose agar (PDA). Macerated tissue was also serially diluted in water and plated on nutrient agar for isolating bacteria.

Pathogenicity tests on harvested carrots. Mature carrot taproots (cv. Imperator) were surface-sterilized in 0.525% NaOCl for 2 min, rinsed in distilled water, and inoculated with six agar plugs (0.5 cm diameter) from 5-day-old cultures of four isolates each of P. violae, P. ultimum Trow, P. irregular tagged Byusman, or P. aphani dermatum (Edson) Fitzg. grown on CMA, or cultures of Alternaria radicina Meier, Drechs. & E. D. Eddy or Rhizoctonia solani Kühn grown on PDA. Two carrots were used for each fungal isolate. Noninoculated carrots received sterile agar plugs. In all treatments, the agar plugs were placed along the longitudinal axis of the taproot about 3 cm apart and covered with wet cotton.
to maintain moisture around the point of inoculation. The carrots were placed in moist chambers (closed plastic containers, 24 × 32 × 10 cm) maintained at 20 C in the dark. After 1–2 wk, the roots were examined for the presence of lesions. Fungi were reisolated from the diseased tissues on CMA or PDA for identification as described earlier. The tests were repeated two times.

Pathogenicity tests on greenhouse-grown carrots. Five isolates of *P. violae* and four isolates of *P. ultimum* were each tested for pathogenicity on growing carrots. All isolates were obtained from cavity spots on carrots collected from commercial fields in Kern County. Five hundred milliliters of vermiculite amended with V8 juice (200 ml of water, 50 ml of V8 juice, and 0.5 g of CaCO₃) was autoclaved twice at 121 C for 45 min and inoculated with five agar plugs (0.5 cm in diameter) from 5-day-old culture of two isolates each of *P. violae* or *P. ultimum* grown on CMA. The vermiculite inoculum was incubated at room temperature (about 25 C) in the dark for 3 wk before receiving carrot seeds or transplants. To determine the inoculum density of *P. ultimum* in the medium, 10 g of inoculum was suspended in 0.2% water agar (1:10,000 dilution, w/v) and plated in 1-ml aliquots on each of three 9-cm-diameter PARP agar plates. After 24 hr at 20 C in the dark, the number of colony-forming units (cfu) per gram of substrate was determined. To estimate the density of *P. violae* in the medium, 10 g of vermiculite was diluted (1:5, w/w) with sterile U.C. mix (1:1, peat/sand) (1), air-dried for 24 hr at 25 C, and pulverized. Aliquots of 0.1 g of the resulting mixture were distributed on each of five PARP plates with an Anderson air sampler. After 36 hr of incubation at 20 C in the dark, the number of colonies was determined. Inoculum of each *Pythium* spp. was diluted with U.C. mix to obtain a final density of approximately 500 cfu/g of soil.

Carrot seeds (cv. Pakmor) were then planted into U.C. mix with or without inoculum of *P. violae* or *P. ultimum* in plastic pots (18.0 cm diameter × 21.25 cm deep) and grown in a greenhouse at ambient temperatures (20–30 C). Six pots of 10 seeds each (later thinned to four plants per pot) were prepared for each treatment. The plants were watered twice daily and fertilized weekly with half-strength Hoagland’s solution (6). After 3 mo, the carrots were lifted from the mix and examined for the presence of lesions, which were surface-sterilized, excised, and plated on PARP agar plates for reisolation of the pathogens.

In addition to the previous experiment, 3-mo-old carrots (cv. Pakmor) grown in the greenhouse were transplanted into plastic bags (540-ml Nasco Whirlpak bags, National Shipping Supply Co., Olate, KS) with drainage holes at the bottom, containing noninfested or *Pythium*-infested U.C. mix. Four bags with three carrots per bag were prepared for each treatment. The bags were arranged in a completely randomized design in a growth chamber at 20 C with 16 hr of light. The carrots were watered twice daily. Four weeks after inoculation, the carrots were lifted from the potting mix, washed, and examined for cavity spot lesions. The pathogens were reisolated from lesions as previously described.

In another experiment, carrot cvs. Topak, Caropak, Pakmor, Sierra, and Dominator were tested for their susceptibility to cavity spot. Three-month-old carrots of each cultivar were transplanted into U.C. mix with or without inoculum of *P. violae* or *P. ultimum* and incubated in growth chambers maintained at 20 C with 16 hr of light. Four pots (18.0 cm diameter × 21.25 cm deep) were each planted with three carrots of each cultivar. Four weeks after inoculation, the carrots were lifted from the potting mix and examined for the presence of lesions. All pathogenicity tests were repeated twice.

Effect of carrot age, soil temperature, and irrigation on cavity spot development. Greenhouse-grown carrots (cv. Pakmor) were transplanted at 3, 4, or 5 mo after planting into plastic bags filled with U.C. mix with or without inoculum of two isolates of *P. violae* or *P. ultimum* (500 cfu/g) and incubated for 4 wk in a growth chamber at 20 C as described earlier. The experiment was repeated once. To study the effect of temperature on cavity spot, 3-mo-old carrots (cv. Pakmor) were transplanted into U.C. mix with or without *P. violae* or *P. ultimum* and incubated in growth chambers maintained at 15, 20, or 25 C. Four weeks after inoculation, the carrots were removed from the potting mix and examined for the presence of lesions. The pathogens were reisolated from lesions on PARP. The experiment was repeated three times. The experimental design was identical to other experiments conducted in the growth chambers.

To study the effect of soil moisture on cavity spot development, 3-mo-old carrots (cv. Pakmor) were transplanted into plastic pots (12.5 cm diameter × 14 cm deep) containing U.C. mix with or without inoculum of *P. violae* or *P. ultimum*. The pots were incubated in a growth chamber maintained at 20 C with 16 hr of light and subjected to three different watering schedules—nonflooded, flood treatment, and 48 or 48 hr of flooding. In the nonflooded treatment, pots were watered as needed (generally once a day) throughout the experiment. In the flooded treatments, the pots were inserted into larger pots (12.5 cm diameter × 16.25 cm deep) without holes and the free water level was maintained 2 cm above the soil surface for 24 or 48 hr. After the flooding treatment, the carrots were watered as necessary for the duration of the experiment. After 4 wk, the carrots were harvested and examined for the presence of lesions. There were four pots containing three carrots each for each treatment. The experiment was repeated twice.

Planting density. To test the effect of planting density on incidence of cavity spot, carrots (cv. Sierra) were planted in the field at the standard planting spacing in the San Joaquin Valley (115 carrots per meter of row) and half or double the standard rate. Treatments were replicated six times in 9.2-m plots arranged in a randomized complete block design in three separate locations in naturally infested commercial fields in Kern County. None of the fields was planted to carrots the previous year. At the end of the season, all the carrots in the center 1 m of each plot were pulled and examined for cavity spot.

Data for all repeated tests, whether by time or location, were combined and analyzed by analysis of variance, and where appropriate, subjected to mean separation or contrast analysis for group comparisons.

RESULTS

Disease survey and isolations. *P. irregulare* and *P. ultimum* were the most abundant *Pythium* species isolated from the soil, accounting for 70% of all isolates. *P. oligandrum* Drechs. and *P. aphanidermatum* accounted for 25 and 3%, respectively. *P. spinosum* Sawada, *P. vexans* de Bary, *P. paroecandrum* Drechs., and *P. catemulatum* Matthews were isolated rarely. *P. violae* and *P. ultimum* were commonly isolated from cavity spot lesions on carrots; no other fungi or bacteria were consistently recovered from cavity spots. *P. solani* was recovered from two cavity spots, whereas *A. radicina* was recovered from only one lesion. *P. violae* was occasionally recovered from the soil by trapping the fungus with carrot seedlings but not by soil dilutions on selective media. Other *Pythium* spp., including *P. ultimum*, were often isolated from the roots of the carrot or alfalfa seedlings.

The incidence of cavity spot ranged from 0 to 72% of the carrots sampled in 1987 and from 1.2 to 27.5% in 1988. Correlations between the incidence of cavity spot and total *Pythium* population (r = 0.17 for both years combined) or individual population densities of *P. ultimum* (r = −0.28 for both years combined) were not statistically significant (P = 0.05).

The minimum and maximum values for the soil characteristics in the 39 fields in 1987 were reported previously (8). In 1988, the range of values for the 15 fields included: pH, 5.7–7.7; electrical conductivity, 0.79–2.82 millimhos/cm; total calcium, 12.6–71.0 meq/100 g; exchangeable calcium, 3.6–22.1 meq/100 g; moisture content at −10 kPa, 7.5–19.0%; moisture content at −1,500 kPa, 3.1–
7.0%; sand, 60–77%; silt, 9–24%; clay, 8–16%; and organic matter, 0.52–1.0%. There were no significant correlations between incidence of cavity spot and values of any of the soil variables in either year. There was a significant (P = 0.05) positive correlation between elapsed time since planting and incidence of cavity spot in the six fields periodically assessed for disease (Fig. 1).

Pathogenicity studies. All isolates of *P. violae* and *P. ultimum* caused typical cavity spot symptoms on carrots seeded into infested U.C. mix. The symptoms were small elliptical lesions with sharp margins. Symptoms also developed on the harvested carrots inoculated with agar plugs of all isolates of *P. violae* and *P. ultimum*, but the lesions generally lacked the sharp margins of typical cavity spots and were superficial. No other organisms tested produced cavity spot symptoms. *P. violae* and *P. ultimum* were recovered on selective media from cavity spot lesions in all pathogenicity tests. Noninoculated carrots were asymptomatic. In growth chamber tests with the 3-mo-old transplanted carrots, all of the plants grown in soil inoculated with *P. violae* and 65% of the carrots inoculated with *P. ultimum* developed lesions. Both fungi were recovered from the lesions.

Cavity spots developed on all the carrot cultivars planted into soil infested with *P. violae* or *P. ultimum*. There were significant differences (P = 0.05) in the number of lesions per carrot caused by either *P. violae* or *P. ultimum* but not in the number of carrots with lesions (Table 1). Of all cultivars tested, Topak was among the most susceptible. Noninoculated carrots were asymptomatic.

**Carrot age, soil temperature, and irrigation.** The number of cavity spot lesions caused by both *P. violae* and *P. ultimum* was positively correlated with carrot age (y = 3.44 + 0.26x, r = 0.88 for the combined data). The 5-mo-old carrots had approximately twice as many lesions as the 3- or 4-mo-old roots. The differences in number of symptomatic carrots, however, were not statistically significant among the three ages. *P. violae* was the more virulent fungus of the two species. Noninoculated carrots were asymptomatic.

**DISCUSSION**

*P. violae* and *P. ultimum* were the fungi most frequently isolated from cavity spot lesions on commercially grown carrots in California. Pathogenicity tests demonstrated that *P. violae* and *P. ultimum* cause cavity spot and that *P. violae* is more virulent on carrots than *P. ultimum*. This may explain the lack of correlation between cavity spot incidence and populations of *P. ultimum* in the field because *P. violae* probably accounts for most of the cavity spot lesions on carrots grown in California.

*P. ultimum*, but not *P. violae*, was also reported to cause carrot root dieback in the San Joaquin Valley (8). In our study, *P. violae* was recovered from cavity spot lesions with variable success, and while it was isolated from soil by trapping the fungus with carrot seedlings, it was not isolated by direct isolation methods on soil dilution plates with selective media. *P. violae* is a slow-growing fungus compared with other *Pythium* species (17), and the soil dilution plates may be overgrown with faster-growing *Pythium* species, such as *P. ultimum* and *P. irregulare*, which are common soil inhabitants in the San Joaquin Valley (8). Additionally, population densities of *P. violae* may be relatively low, which further prevents easy separation from other *Pythium* species with much higher population densities. For optimum recovery of *P. violae* from carrot tissues, our experience showed that the carrots must be kept cool during transport to the lab, and isolations should be made within 1–3 days. Old cavity spot lesions are often overrun with secondary fungi and bacteria, complicating isolation of *P. violae* from carrot tissues.

The symptoms observed on carrots grown in potting mix artificially infested with *P. violae* or *P. ultimum* were typical of the cavity spot lesions seen in the field, whereas lesions on mature carrots inoculated with...
Table 2. Effect of soil moisture on the number of lesions of cavity spot caused by *Pythium violae* and *P. ultimum*.

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Flooding period (hr)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td><em>P. violae</em></td>
<td>21</td>
</tr>
<tr>
<td><em>P. ultimum</em></td>
<td>9</td>
</tr>
<tr>
<td>Contrast</td>
<td></td>
</tr>
<tr>
<td>Flooded vs. nonflooded</td>
<td>1,202.014*4</td>
</tr>
<tr>
<td>Flooded 24 hr vs. 48 hr</td>
<td>0.427*7</td>
</tr>
<tr>
<td><em>P. violae</em> vs. <em>P. ultimum</em></td>
<td>1,519.880*2</td>
</tr>
</tbody>
</table>

*Cavity spot incidence on 3-mo-old carrots transplanted into soils inoculated with *P. violae* or *P. ultimum* and maintained for 4 wk in a growth chamber at 20 C.

*In number of lesions per carrot.

*Each value is the mean of four replications of three carrots.

*Mean square.

*P = 0.01.

lated in the laboratory with agar plugs after harvest were superficial, discolored areas with indistinct margins. The appearance of these atypical blemishes suggested that the harvested carrots were unable to respond to fungal attack with the characteristic elliptical, sharp-edged lesions. Thus, screening for resistance to cavity spot by placing inoculated agar plugs on harvested, surface-sterilized carrots may not be an accurate technique.

Incidence of cavity spot was not significantly correlated with the range of total or exchangeable calcium in fields included in this study, contrary to an early report that explained the cause of cavity spot as calcium deficiency (10). Also, we did not find that cavity spot incidence was reduced in fields with a relatively high pH. The average pH of fields in Kern County was 7.4, and cavity spot was observed in most of these fields. In Great Britain, cavity spot was almost absent in fields with a pH of 7.4–8.0 and above (14,20).

Cavity spot was more severe at 15 C than at 20 or 25 C. Similar results were reported by Monfort and Rouxel (12) for *P. violae* in laboratory conditions. In the San Joaquin Valley of California, average soil temperatures are 15 C or below at 15 cm of depth during November–March (5), the period of time when cavity spot is most often observed in California and carrot production is most intense.

The carrot cultivars included in the growth chamber tests were planted in 68% of the fields during the 2-yr survey. All were susceptible to cavity spot. All cultivars examined originated from few parental lines, and the lack of genetic diversity may explain the similar responses to *P. violae* and *P. ultimum*. The lack of resistance to cavity spot also was observed in the United Kingdom (3,16,20) and in Israel (15). Currently, none of the carrot cultivars used in California appear to be resistant to cavity spot based on our tests and on field observations. However, the small differences in tolerance observed in some cultivars suggest that breeding, and possibly the introduction of new parental lines, may eventually lead to tolerant cultivars.

The incidence of cavity spot was greatest on older carrots in the field and in the growth chamber experiments. This increased level of disease could be a result of increased susceptibility as carrots mature, an accumulation of lesions over time, or an expansion of lesions as the diameter of the carrot increases. Soil compaction surrounding the expanding carrot may also result in changes in oxygen relationships, which may influence disease progress. Wagenvoort et al. (18) reported that disease symptoms were more visible during the second part of the growing season. They suggested a relationship between carrot age and cavity spot appearance and an increase in the chance of infection as the carrot root surface increases. Root area and time of root exposure to infection may play a role in lesion development, but changes in root physiology also may increase susceptibility. An increase of susceptibility with age also was observed by Groom and Perry (3) and by Sweet et al. (16).

Our results are consistent with those reported from Europe where *P. violae* also was implicated as the primary cause of cavity spot of carrots (3,12,19,20). Although relatively little is known about this fungus, carrots are apparently readily attacked in different regions of the world. To develop optimum control strategies for reducing cavity spot incidence, more research is needed on the host range of *P. violae*, the nature of its surviving propagule, and the mechanism of infection, among other factors.

**LITERATURE CITED**


